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Genetic and Cellular Effects of Microwave Radiations

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16. ABSTRACT

This research program was initiated with the overall objective of determining genetic and cellular effects from exposure of unicellular organisms to selected frequencies of CW and pulsed microwave radiation which is prevalent in our biospnere.

Several tester strains of the bacterium Salmonella typimurium, TA-98, TA-100, TA-1535 and TA-1538; the bacterium Escherichia coli, W3110 (pol A^+) and p3438 (pol A^-, repair deficient); and the yeast Saccharomyces cerevisiae, D_3, D_4 and D_5 were tested for lethal amd mutagenic events. Effects of known elevated temperatures were studied to distinguish microwave induced temperature effects from the direct temperature effects.

Three kinds of microwave exposure systems were used in these studies: (1) farfield antenna (for 2.45 GHz and 8.5 - 9.5 GHz), (2) waveguide (for 8 - 10 GHz) and (3) TEM (transverse electric and magnetic mode) transmission lines for 915 MHz radiation. The SAR (specific absorption rate) for various exposures ranged from 0.1 W/kg to 40 W/kg. Pulse repetition rates were 400 Hz and 1000 Hz for pulsed microwave radiations.

The studies revealed no increase in mutations or of gene conversions wnen cells were exposed to microwave radiations, but yeast and bacterial strains showed cellular lethality caused by temperature rises (greater than 10°C) at higher power levels.

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GENETIC AND CELLULAR EFFECTS OF MICROWAVE RADIATIONS

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FOREWARD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report is directed at the question: Does radiofrequency radiation cause mutagenic changes in biological entities? Simple unicellular organisms are used to examine the mutagenic potential of several specific frequencies of non-ionizing electromagnetic radiation.

F. Gordon Hueter
Director
Health Effects Research Laboratory

ABSTRACT

This research program was initiated with the overall objective of determining genetic and cellular effects of CW and pulsed microwave radiations which are prevalent in our biosphere.

Several tester strains of the bacterium Salmonella typhimurium, TA-98, TA-100, TA-1535, and TA-1538; the bacterium Escherichia coli W3110 (pol A^+) and p3438 (pol A$^-$, repair deficient); and the yeast Saccharomyces cerevisiae D_3, D_4, and D_5 were tested for lethal and mutagenic events when exposed to microwave radiations. Effects of known elevated temperatures were studied to distinguish microwave-induced temperature effects from the direct temperature effects.

Three kinds of microwave exposure systems were used in these studies: (1) far-field of an antenna (for 2.45 GHz and 8.5-9.5 GHz), (2) waveguide (for 8-10 GHz), and (3) transmission line (TEM cell) for 915 MHz radiation. The SAR (specific absorption rate) for various exposures ranged from 0.1 W/kg to 40 W/kg. Pulse repetition rates were 400 Hz or 1000 Hz for pulsed microwave radiations.

The studies revealed no increase in mutations or of gene conversions when cells were exposed to microwave radiations, but yeast and bacterial strains showed cellular lethality caused by temperature rises at higher microwave power densities. Results demonstrated that very high elevated temperatures (above 10°C rise) generated by the microwave exposure could produce genetic events in microbial assay systems. At a SAR of 12 W/kg, an indication ($0.05 < P < 0.1$) of microwave-induced increase in cell concentration in terms of increased colony forming units (CFU) was observed in the E. coli pol A$^-$ strain at 8.6 GHz when exposed for more than seven hours. No change in CFU were noticed when E. coli cells were exposed to 915 MHz, 8.6, 8.8, or 9.0 GHz at very low power densities (SAR of 1 W/kg). At 10 GHz, E. coli cells were exposed at varying power levels (SAR of 0.1, 1, or 10 W/kg). No change in CFU was observed.

Mathematical expressions were developed using a SAS-76 computer program to predict the concentration of bacterial cells (CFU) at any time within the temperature range 37°C-49°C and to measure microwave-induced changes in CFU as a function of temperature rise. A heat transfer model was developed for our exposure systems which helps to distinguish low power density effects when a temperature rise due to microwave radiations cannot be detected.

This report was submitted in fulfillment of the Grant No. R-803561 by Howard University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the work period during May 8, 1975, to December 31, 1979.

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SECTION 1

INTRODUCTION

Due to their widespread industrial and domestic uses in heating and drying equipment, spectroscopy, alarm system, diathermy units, radar, TV transmission, communications, and navigation devices, microwave radiations have become an environmentally important energy polluter. The effects of higher intensities of microwave radiation on test systems in vivo and in vitro have been investigated by various groups (Cleary, 1977). These studies have revealed effects of microwave radiation on (a) the lens of the eye, (b) the gonads, (c) embryonic development, (d) chromosome aberrations, and (e) electrophoretic and immunologic properties and enzymatic activity of isolated proteins. The results of the in vivo studies generally reflect the physiological responses of the test organism to the thermal burden imposed by microwaves. In contrast, a number of Soviet and East European investigators have reported low level microwave effects at the cellular level (Baranski and Czerski, 1976).

Many of these Soviet works are primarily on effects on cell membrane, biological macromolecules, tissues, and growth inhibition of unicellular organisms (Baranski and Czerski, 1976). The potential hazard of such radiation might escape detection if investigations are not conducted simultaneously with a battery of highly sensitive genetic and morphological tests using a spectrum of genetic system--both unicellular and multicellular haploid and diploid microorganisms. The biological action of many poorly understood physicochemical phenomena produced by microwave irradiation may conceivably include such long term hazards as (a) recessive mutations giving rise to genetic diseases and (b) cumulative effects of deletion mutations that may go undetected for several generations.

Studies on genetic effects of microwaves have been cited as a key priority area in accordance with the policy report of the Office of Telecommunications (see report on NEMR U.S. Government Presidents Executive Office, May, 1974). It is known that a battery of microbial tester strains are quicker and far more sensitive for detecting minute genetic differences than are higher animals. In addition, tests using higher animals are extremely expensive and time consuming, taking several generations before meaningful results are obtained.

SECTION 2

CONCLUSIONS

In accordance with the objectives of the project, major thrusts were two-fold: (1) to detect any minute changes in the genes at the nucleotide level and (2) to identify any non-genetic (cellular) effects of microwave radiations. Conclusions arrived for these objectives are as follows:

The extensive genetic studies made with a wide variety of microbial tester strains revealed no increase in mutations or in mitotic gene conversions when the strains were exposed to low or moderate levels of microwave radiation in a far-field exposure system. However, at higher power levels, yeast and bacterial strains showed cellular lethality caused by a temperature rise. These results demonstrated that very high elevated temperatures (above 10°C rise) generated by microwave exposure could produce genetic events in microbial assay systems.

Our studies on cellular effects were based on counts of colony forming units (CFU) of treated and control (sham) experiments. Using a waveguide exposure system, having precise temperature control, the colony forming units in an exposed sample of E. coli, pol A strain, was observed to increase slightly ($0.05 < P < 0.1$). In this case, exposures were conducted for more than seven hours using 8.6 GHz pulsed radiation at a SAR of 12 W/kg which had a pulse width of 1 μsec and a pulse repetition rate of 1 kHz.

No radiation-induced change in CFU was noticed when E. coli cells were exposed to 915 MHz, 8.6, 8.8, and 9.0 GHz at very low levels (SAR of 1 W/kg). At 10 GHz, E. coli cells were exposed at varying power levels (SAR of .1, 1, or 10 W/kg). No change in CFU was noticed. Both waveguide and Crawford cell exposure systems were used.

As a part of our attempt ot obtain some fundamental information on common problems associated with microwave research, we did extensive studies on the effects of elevated temperature. The mathematical expressions were developed using a SAS-76 computer program to predict cell concentrations in terms of colony forming units (CFU) in bacteria at any time within the temperature range 37°C-49°C and to measure microwave-induced CFU as a function of temperature rise. A heat transfer model was developed for our exposure system which helps to distinguish low power density effects when temperature rise due to microwave radiations cannot be detected within the biological system.

SECTION 3

RECOMMENDATIONS

There is conclusive evidence for definite cellular effects, but no genetic damage, from extremely low intensity microwave radiations. Because of the evidence for cellular effects in prokaryotic and lower eukaryotic systems, we strongly recommend that studies be performed on complex eukaryotes, specifically human cells in culture, to investigate the effects of this radiation on a potentially more labile system which has direct human relevance.

(1) The existence of power and frequency "windows" which has been reported to affect ions associated with brain tissue should be studied more extensively. The well-established human neuroblastoma cell line AG2202 can be used as a cellular system for Ca^{++} efflux studies at several frequencies and different power levels to rapidly examine these responses.

(2) Once the frequency and power density "windows" have been established for neuroblastoma cells in culture, these values should be used to examine the response of other human cell lines, such as IMR-90, established from normal tissue. These experiments should be conducted with very careful attention to the effects of microwave-induced heating in order to examine other possible causes of microwave-induced biological changes. Computerized mathematical expressions of cell survivals and/or concentration increases as a function of temperature rises will be used to distinguish microwave effect from temperature effects.

(3) After basic information regarding power and frequency "windows" are obtained, primary explants in culture from skins of human cadavers should be exposed to see the microwave-induced effect on tissue from the human body. Studies should involve histological changes like necrosis or any gross morphological change and/or growth inhibition using ^{3}H-thymidine incorporation procedure to detect abnormality in rate of mitosis. Electron microscopic studies can be performed to study any changes in membrane structure.

SECTION 4

MATERIALS, METHODS, AND PROCEDURES

MICROBIAL TESTER STRAINS

Table 1 outlines all strains used in our various studies and their relevant markers and references to their use in genetic screening. The procedures for maintenance and culturing and the methodology for identification and quantitation of changes in genetic and non-genetic events have been described by Brusick and Mayer (1973), Ames et al. (1975), and Zimmermann (1975).

Diploid strains of Saccharomyces cerevisiae designated D_3, D_4, and D_5 were obtained from Dr. F. K. Zimmermann, Technische Hoschschule, Dārmstadt, German Federal Republic. These three strains were established by Zimmermann. Strains D_3 and D_4 detect mitotic recombination and mitotic gene conversion, respectively, whereas strain D_5 detects mitotic recombination and probably mitotic gene conversion events simultaneously. Mitotic events of D_3 and D_5 are identified by the production of red and/or pink pigment associated with mutant alleles of the ade 2 locus. Gene conversion is identified in strain D_4 heteroalleles at both ade 2 and trp 5 loci which utilize adenine and tryptophan selective media.

The bacterial strains of Salmonella typhimurium designated TA-1535, TA-1538, TA-98, and TA-100 were obtained from Dr. B. N. Ames, University of California. All of the S. typhimurium strains were scored by numbers of revertants in histidineless minimal medium. Strains TA-1535 and TA-100 are basepair substitution mutants whereas TA-1538 and TA-98 are frameshift mutants.

Escherichia coli strains designated pol A^+ and pol A^- were obtained from Dr. H. Rosenkranz, Columbia University, NY. The mutant strain pol A^- is deficient in DNA polymerase and is exceedingly sensitive to Uv radiation, radiomimetic agents and agents known to react with cellular DNA (D'Alisa et al., 1971; DeLucia and Cairus, 1969; Gross and Gross, 1969). This repair-deficient mutant of E. coli is very sensitive to nonspecific damange in DNA which is repairable in normal pol A^+ cells but which results in cell death in repair-deficient pol A^- cells. The purity of these two cultures was tested regularly as follows: 0.1 ml portions of each strain were spread separately on the surface of standard agar plates. The plates were left at room temperature for two hours. One tenth ml of methyl methane sulfonate (MMS) was then poured at the center of each plate which was then placed in the incubator at 37°C for 16 hours. A larger zone of inhibition exhibited by pol A^- indicated the purity of the culture.

TABLE 1. DESCRIPTION OF MICROBIAL STRAINS EMPLOYED

Name of the organism	Strain designation	Gene affected	Additional Mutations			References for methodology
			Repair	LPS	R factor	
Salmonella typhimurium	TA-1535	*his* C	*uvr* B	*rfa*	-	Ames et al., 1975
	TA-98	*his* D	*uvr* B	*rfa*	pKM101	Ames et al., 1975
	TA-100	*his* G	*uvr* B	*rfa*	pKM101	Ames et al., 1975
Escherichia coli	W3110	-	Normal	-	-	Slater et al., 1971
	p3478	*pol* A	No excision	-	-	Slater et al., 1971
Saccharomyces cerevisiae	D3	*ade* 2, *his* 8	Normal	-	-	Zimmermann et al., 1965
	D4	*ade* 2, *try* 5	Normal	-	-	Zimmermann and Schwaier, 1967
	D5	*ade* 2	Normal	-	-	Zimmermann, 1975

CULTURE MEDIA AND GROWTH CONDITIONS

The composition of complete broth medium for yeast has been described by Brusick (1970), and the composition of minimal medium has been described by Magni and Von Borstel (1962). Adenine and tryptophan were added to minimal medium where appropriate at a concentration of 10-20 mg percent.

The composition of complete broth medium for growing bacterial (Salmonella and E. coli) cultures was Difco Standard Methods Broth (Difco, Detroit, MI), and the composition of minimal medium was that of Spizizen (1958).

Each bacterial strain was cultured in complete broth at 37°C for 16 to 20 hours. One hour prior to exposure at a specific temperature, each culture was diluted to an optical density (OD) of 0.25 at 610 nm in order to provide between 100 and 300 colonies per plate. All cultures were maintained for one hour at the appropriate temperature on a shaker to allow for post-adjustment growth. Just prior to treatment, cell concentration was determined for each sample by plating on complete medium.

MICROWAVE EXPOSURE SYSTEMS

Far-field

Exposure to 2.45 GHz microwaves took place in an electrically anechoic, chamber-type far-field exposure facility that provides linearly polarized, regulated CW radiation (Elder and Ali, 1975; Blackman et al., 1975). The X-band exposures also took place in an anechoic chamber-type system with the following major components: an Applied Microwave Lab Model MH250 Pulse Modulator, a Raytheon RK2J51 magnetron, a Systron Donner Model DBH-250 10 dB horn antenna, and a Vista Scientific environmental control system. Samples were exposed to linearly polarized, rectangularly pulsed RF radiation at frequencies between 8.5 and 9.6 GHz with a pulse repetition rate of 1000 Hz and a duty cycle of 0.001. Both facilities have environmental control systems that maintain temperature and humidity at present levels (50% ± 5% relative humidity and an air velocity of 5.4 m/min). One chamber, located in the anechoic room, houses the sample to be exposed while the other chamber houses the control sample. Temperature of the exposure and control chambers was maintained as appropriate for the test systems. Figure 1 is a block diagram of the X-band system.

Waveguide Exposure System

Details of the equipment arrangement for the waveguide exposure system are depicted in Figure 2 and described in details by Ho (1976). The pulsed wave had a width of 1 μsec and a pulse repetition rate of 1 KHz. One ml of each culture in broth was placed in a metallic holder

FIGURE 1. Block diagram for far-field antenna system (X-band).

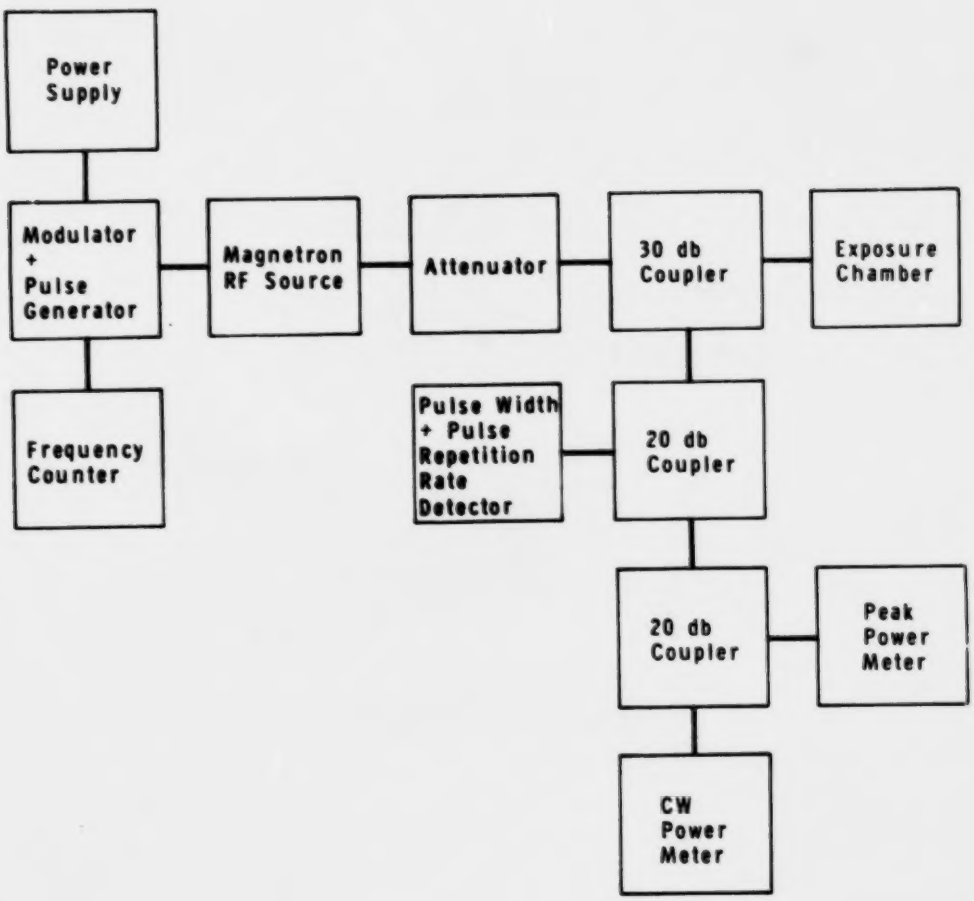
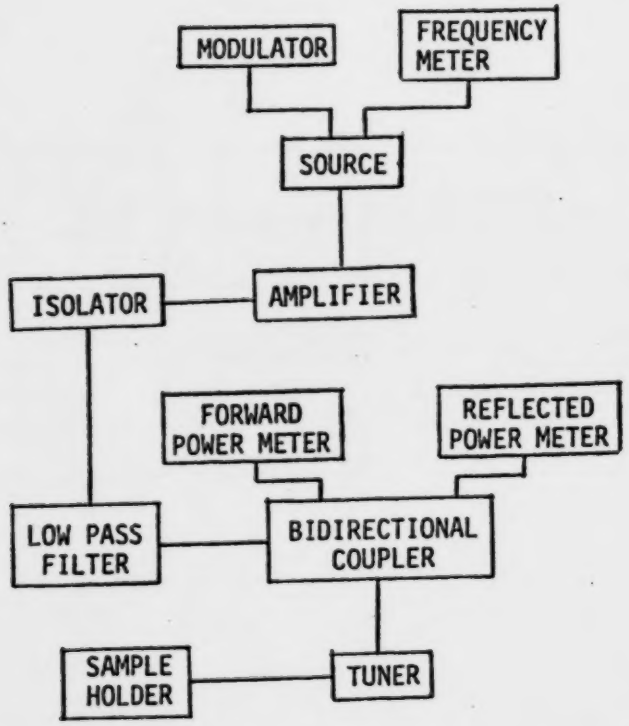


FIGURE 2. Block diagram for waveguide EMR system.



which was constructed from a piece of waveguide terminated by a shorting plate. The holder was connected to the system as diagrammed in Figure 2. The holder was immersed in a water bath at 37° ± 0.1°C. Another holder of the same material containing another 1.0 ml aliquot of the culture was also immersed in the bath to serve as the sham exposure. The holders were shaken frequently so that the cells remained in uniform suspension. This shaking was accomplished without interrupting exposure of the samples.

Crawford Cell Microwave Exposure System

The Crawford cell originally conceived at the National Bureau of Standards, now being used at the EPA Laboratory (Instruments for Industry, Model CC105S), consists of a rectangular TEM (transverse electric and magnetic) mode transmission line tapered at each end to a transition which mates with a standard coaxial cable. This test cell offers a new and very broad-band method of measuring the absorbed power of the sample placed within the cell. It offers an extremely efficient means of obtaining broad-band operation up to approximately 1.0 GHz. Figure 3 is a block diagram for this system.

PROCEDURE FOR ESTIMATION OF SPECIFIC ABSORPTION RATE (SAR)

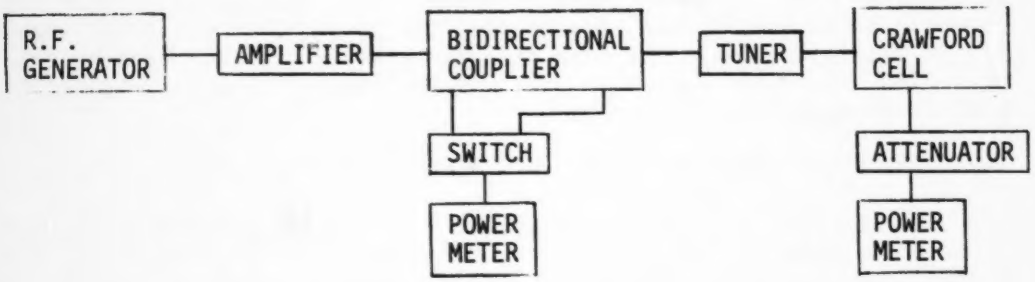
The specific absorption rate (SAR) in a far-field system was determined at selected intensities and frequencies by the procedures of heating and cooling curve analysis described by Allis et al. (1977). In order to determine the amount of power absorbed in the waveguide exposure system by bacterial cells and medium, both the incident and reflected power meters were monitored. The reflected power was minimized by adjusting the tuner. The power absorbed by the bacterial culture was maintained at a definite SAR. Details of the procedures are given by Ho (1976).

The field generated inside the Crawford cell chamber may be calculated from the expression $E = V/D$, where E is the field generated in volts per meter, V is the input voltage to the cell, and D is the septum to top plate separation in meters. Thus, when the sample is placed inside the chamber and exposed to microwave, the amount of power absorbed within the sample will be $Pi - (Pr + Pt)$ where:

Pi, the incident power;
Pr, the reflected power; and
Pt, the transmitted power.

In order to monitor the temperature of the exposure chamber, the Crawford cell will be placed inside a versatile temperature and humidity controlled room. The temperature in the chamber can be varied over the range of 18 to 42°C to within ± 0.1°C.

FIGURE 3. Block diagram of the TEM Crawford cell microwave exposure system.



EXPOSURE OF CELLS TO ELECTROMAGNETIC RADIATION AND SUBSEQUENT ASSAYS

Log-phase of yeast cells cultured in broth were adjusted to a cell density of 10^7 cells/ml and were used as stock for subsequent treatments. Prior to treatment, cultures were shaken for 60 minutes at 30°C. Log-phase bacteria were grown in complete broth, centrifuged, and then resuspended in fresh broth to densities of 10^9 cells/ml; the suspension was then shaken for 90 minutes at 37°C. This suspension served as the stock bacterial culture. The yeast suspensions were exposed for 2 hours to 2.45 GHz radiation at 30.0 ± 0.5°C and to X-band radiation at 29.0 ± 0.5°C. Following exposure, yeast cells were plated on complete medium at a dilution ratio of 10^{-5} and on selective media either undiluted or at a dilution ratio of 10^{-1}. Scoring was performed after 72 hours and the number of convertants per 10^5 surviving cells was calculated.

The bacteria were exposed for 90 minutes at 37.0 ± 0.5°C in the 2.45 GHz facility and at 35.0 ± 0.5°C in the X-band facility. In both facilities, the samples were irradiated from above. Zero-hour samples of bacteria and yeast were plated to determine the initial conditions of the cultures and to provide a basis for calculating the number of generations of growth that occurred during treatment. Bacterial cultures were plated on complete medium at a dilution ratio of 10^{-7} to determine the total number of colony forming units per ml and undiluted to determine the number of revertants per survivor. Scoring was performed after 48 hours of incubation and is expressed as revertant colonies per 10^8 survivors.

After exposures, each *E. coli* sample was plated in 0.1 ml aliquots on each of five plates (Standard Methods Agar, SMA, Difco, Detroit, MI). The plates were incubated at 37°C for 24 hours and the total number of colonies counted. Each such experiment was repeated three times for every exposure condition. The initial concentration of cells for exposure and sham was between the range of 2.2 and 3 x 10^8 cells/ml. Control experiments were performed by placing cultures in 35 mm circular petri plates at 37°C.

EXPOSURE OF CULTURES TO ELEVATED TEMPERATURES

Cultures of bacteria and yeast were exposed to various ambient temperatures up to 15°C above their optimum growth temperature in Thelco incubators which maintained the set point temperature within ± 0.3°C. Three ml cell suspensions from each culture (both in saline and in broth) were placed in individual 35 mm diameter petri plates and incubated for either 2 hours for *S. typhimurium* and *Sacch. cerevisiae* or for 1, 5, 10, or 15 hours for *E. coli*.

The preparation of quiescent cultures was different for exposure in saline (0.9 percent). Overnight cultures of both strains of *E. coli* were first washed two times in saline and then resuspended in saline to an optical density of 0.20 at 610 nm before being placed on the exposure vessel.

POSITIVE CONTROLS AND DATA ANALYSIS

Genetic Index

Positive control experiments were conducted with all the microbial tester strains using the known chemical mutagen, ethyl methanesulfonate (EMS). This was done to ensure that the organisms were responding at the proper level of sensitivity in these experiments. The results were consistent with published data (Brusick and Andrews, 1975; Ames et al., 1975; Slater et al., 1971).

Effects of elevated temperatures and pulsed microwave radiation on Sacch. cerevisiae and on S. typhimurium were expressed as frequency of genetic events per surviving cell population. Based on positive control studies and on historical control data from the literature, the following method was selected to summarize the data: genetic-activity index = frequency of genetic events in a treated population divided by the frequency of genetic events in a control population (Dutta et al., 1979). If there were no radiation-induced effects, the genetic-activity index would be 1.0 or less. If there were induction of mutations or mitotic recombination, then the frequency of either in the treated population would be higher than that of the control population, and the genetic-activity index would be greater than 1.0. Based on previous observations of normal variation in this system, all genetic-activity index values between 1.0 and 2.0 are considered to be within the range of normal fluctuation. Values greater than 2.0 are considered "suspect," and values greater than 3.0 are considered positive.

CALCULATION OF REPAIR INDEX

The data for E. coli pol A^- were expressed as a repair-index which is defined as the ratio of the percent survival of the repair deficient mutant to the percent survival of the normal strain when both are exposed under the same conditions. Theoretically, a repair index of less than one indicates repairable DNA damage caused by the treatment. However, because of normal fluctuations in growth normally encountered in our laboratory, we have considered repair index values less than 0.85 to be positive.

Repair Index (R.I.) is defined as

$$R.I. = \frac{N_-^I}{N_-^C} \Big/ \frac{N_+^I}{N_+^C} \qquad (1)$$

where N_-^I and N_+^I are the number of irradiated cells of E. coli pol A^- and pol A^+, respectively, and N_-^C and N_+^C are the number of unexposed cells (control) of pol A^- and pol A^+, respectively.

The repair index can also be expressed as a function of time. Since the growth of the cells at 37°C in nutrient broth is exponential, the number of cells N at any time t can be expressed as

$$N = N_o e^{kt} \tag{2}$$

where N_o = the number of cells at time t = 0 and k = a constant representing growth which is temperature dependent. Substituting Eq. (2) into Eq. (1)

$$R.I.^{I} = \frac{e^{k_-^{I} t}}{e^{k_-^{C} t}} \Big/ \frac{e^{k_+^{I} t}}{e^{k_+^{C} t}} \tag{3}$$

The superscripts (C,I) on k indicate the k values for control and irradiated samples and subscripts (+,-) on k values stand for pol A^+ and pol A^-, respectively. The k values are obtained by regression analysis of the experimental data.

A similar expression for the repair index of sham exposed culture can be written as

$$R.I.^{sh} = \frac{e^{k_-^{sh} t}}{e^{k_-^{C} t}} \Big/ \frac{e^{k_+^{sh} t}}{e^{k_+^{C} t}} \tag{4}$$

where the subscript (sh) stands for sham.

SECTION 5

RESULTS

LACK OF MICROBIAL GENETIC RESPONSE

Strain D_4 of the yeast Sacch. cerevisiae and strains TA-1535, TA-100, and TA-98 of the bacterium S. typhimurium were exposed to 2.45 GHz continuous wave or 8.5-9.6 GHz pulsed electromagnetic radiation (EMR) at various power densities from 1 to 45 mW/cm^2. The temperature during radiation was maintained at 30°C for yeast cultures and at 37°C for bacterial cultures. The studies revealed no increase in mutations or of mitotic gene conversions when cells were radiated for two hours or less. Decreased viability of cells was noted in all cultures tested after radiation at power densities of 30 mW/cm^2 or more; however, no reliable changes in genetic events occurred. Results are summarized in Tables 2 through 6.

TABLE 2. EFFECT OF RADIATION AT DIFFERENT POWER DENSITIES ON CELL SURVIVAL AND GENETIC ACTIVITY OF SACCH. CEREVISIAE STRAIN D_4 (9.0 GHz PULSED EMR)

mW/cm^2	% Survival	Gene convertants/10^5 survivors ade + Exposed	ade + Sham	tryp + Exposed	tryp + Sham	Genetic activity index ade +/tryp +
1.0	96	5.6	5.7	1.2	1.2	1.08/1.0
5.0	100	7.1	7.6	2.5	2.1	0.93/1.15
8.9	78	8.4	8.2	2.7	1.9	1.02/1.37
10	95	4.4	5.5	8.0	7.4	0.79/1.07
15	100	-	-	3.0	1.5	- /0.82
30	79	2.2	3.6	4.4	4.3	0.61/1.01
35	88	4.0	5.3	2.2	4.6	0.75/0.49
40	88	36.0	42.0	2.8	2.0	0.85/1.38
45	79	48.0	41.0	3.4	1.9	1.17/1.77

TABLE 3. EFFECT OF DIFFERENT POWER LEVELS AND EMR FREQUENCIES ON CELL SURVIVAL AND GENETIC ACTIVITY IN SACCH. CEREVISIAE STRAIN D_4

Frequency (GHz)	% Survival Power densities/cm² 1mW	5mW	45mW	Genetic activity index 1mW ade+/trp+	5mW ade+/trp+	45mW ade+/trp+
8.5	93	100	55	1.41/1.50	0.76/0.80	0.60/0.63
8.6	88	99	62	1.13/0.92	1.20/1.11	1.27/1.37
8.8	77	78	59	1.27/1.62	1.18/0.95	1.90/1.00
9.0	96	100	79	0.92/1.02	1.08/1.15	1.16/1.16
9.2	-	99	-	1.88/1.42	0.91/1.94	1.49/1.24
9.4	92	100	82	1.62/1.24	0.91/1.79	2.17/0.97
9.6	74	100	42	1.14/1.12	0.83/0.83	1.00/1.48

TABLE 4. EFFECT OF PULSED EMR RADIATION AT DIFFERENT FREQUENCIES AND POWER LEVELS ON S. TYPHIMURIUM STRAIN TA-100

Power mW/cm²	Percent survival	Frequency in GHz	Reversion/10^8 cells Irradiated	Sham	Genetic activity index
10	90	8.6	40	33	1.20
10	100	8.8	27	17	1.38
10	100	9.0	31	27	1.16
10	100	9.4	35	25	1.36
10	88	9.6	28	44	0.61
45	82	8.5	42	35	1.20
45	70	8.6	45	34	1.32
45	82	8.8	59	30	1.91
45	84	9.0	47	20	0.43
45	80	9.2	48	47	1.01
45	86	9.4	28	36	0.78
45	79	9.6	41	33	1.23

TABLE 5. EFFECT OF PULSED EMR RADIATION AT DIFFERENT FREQUENCIES ON S. TYPHIMURIUM STRAIN TA-1535 WHEN EXPOSED AT 10 mW/cm^2

Frequency in GHz	Percent survival	Reversion/10^8 cell		Genetic activity index
		Irradiated	Sham	
8.6	96	57	48	1.19
8.8	92	27	29	0.93
9.0	100	88	34	2.57
9.4	100	94	66	1.43
9.6	80	23	26	0.87

TABLE 6. EFFECT OF PULSED EMR RADIATION AT DIFFERENT FREQUENCIES ON S. TYPHIMURIUM STRAIN TA-98 WHEN EXPOSED AT 10 mW/cm^2

Frequency in GHz	Percent survival	Reversion/10^8 cell		Genetic activity index
		Irradiated	Sham	
8.6	100	324	210	1.55
8.8	95	207	150	1.38
9.0	100	830	432	1.91
9.4	94	122	293	0.42
9.6	100	170	420	0.48

CELLULAR EFFECTS OF ELEVATED TEMPERATURES AND MICROWAVES

Effects in Far-field Exposure

The tester strains of S. typhimurium, E. coli, and S. cerevisiae were examined for lethal events when exposed to elevated temperatures or to X-band, pulsed microwave radiation at various power densities. When compared to E. coli pol A$^+$ under growing conditions, E. coli pol A$^-$ exhibited decreased cell growth when exposed to microwave radiation at power levels at or above 20 mW/cm^2 as well as to temperature levels above 42°C. All yeast and other bacterial strains showed cellular lethality at similar microwave intensities and elevated temperatures. When exposed to elevated temperatures in saline, both quiescent yeast and Salmonella strains exhibited lethal events. However, the Salmonella strains tested showed comparatively less induction of genetic events in the quiescent state compared to induction when the cells were actively growing in both. These results demonstrate that elevated temperatures generated by microwave exposure could produce genetic events in microbial assay systems. If such systems are to be of value in examining the nonthermal genetic potential of microwave radiation, careful control over exposure conditions will be required to eliminate heat-induced genetic events. Results are summarized in Tables 7 to 9 and Figure 4.

TABLE 7. EFFECT OF ELEVATED TEMPERATURES ON DIFFERENT STRAINS OF SACCHAROMYCES CEREVISIAE WHEN EXPOSED FOR 2 HOURS IN SALINE

Strain	Temp. °C	% Survival	Events* /10^5 Cells
D_3	30	100	15
	33	74	20
	40	68	15
	45	23	45
D_4	30	100	5/3
	33	84	2.5/3
	40	50	6/5.5
	45	21	-
D_5	30	100	89
	33	89	91
	40	65	105
	45	19	150

*The scores of genetic events were done as per the following criteria:

D_3 = Frequency of red$^+$ colonies or sectors.
D_4 = Frequency of ade$^+$ or try$^+$ convertants expressed as a ratio of ade/try.
D_5 = Frequency of red/pink, red or pink sectors.

TABLE 8. EFFECT OF ELEVATED TEMPERATURES ON DIFFERENT STRAINS OF SALMONELLA TYPHIMURIUM AFTER 2 HOURS TREATMENT

Strain	Temperature	Broth		Saline	
		% Surv.	Events/10^8 Cells	% Surv.	Events/10^8 Cells
TA-1535	37	100	7	100	1
	42	83	15	78	1
	47	63	10	18	2
	52	No Survivals			
TA-100	37	100	20	100	7
	42	92	21	90	6
	47	60	30	23	16
	52	No Survivals			
TA-1538	37	100	5	100	5
	42	63	11	96	6
	47	14	37	21	16
	52	No Survivals			
TA-98	37	100	78	100	4
	42	60	310	75	3
	47	20	367	8	40
	52	No Survivals			

The strain TA-98 is extremely sensitive (spontaneous rate being $60/10^8$ cells instead of $5\text{-}20/10^8$ cells in other strains) because of an added episome.

TABLE 9. EFFECTS OF DIFFERENT TEMPERATURES AND DURATIONS OF EXPOSURE ON REPAIR INDICES (AVER) OF E. COLI POL A^- RELATIVE TO POL A^+

Temp. °C	Hours Exposed				Times Repeated
	1	5	10	15	
37	1.00	1.00	1.00	1.00	Baseline
40	1.14 ± .06	0.98 ± .03	0.90 ± .07	0.95 ± .07	3
43	1.08 ± .07	0.96 ± .10	0.79 ± .09	0.62 ± .05	3
46	0.95 ± .03	0.82 1 .05	0.48 ± .11	N.S.	3
49	0.34 ± .02	0.11 ± .08	N.S.	N.S.	5

In each experiment, both E. coli pol A^+ and pol A^- strains were exposed to 37°C which was used as the baseline (i.e., 100% survival) temperature. Repair indices (R.I.) were calculated by using the standard formula:

$$\text{R.I.} = \frac{\text{Survival \% of pol } A^-}{\text{Survival \% of pol } A^+}$$

N.S. = No Survival

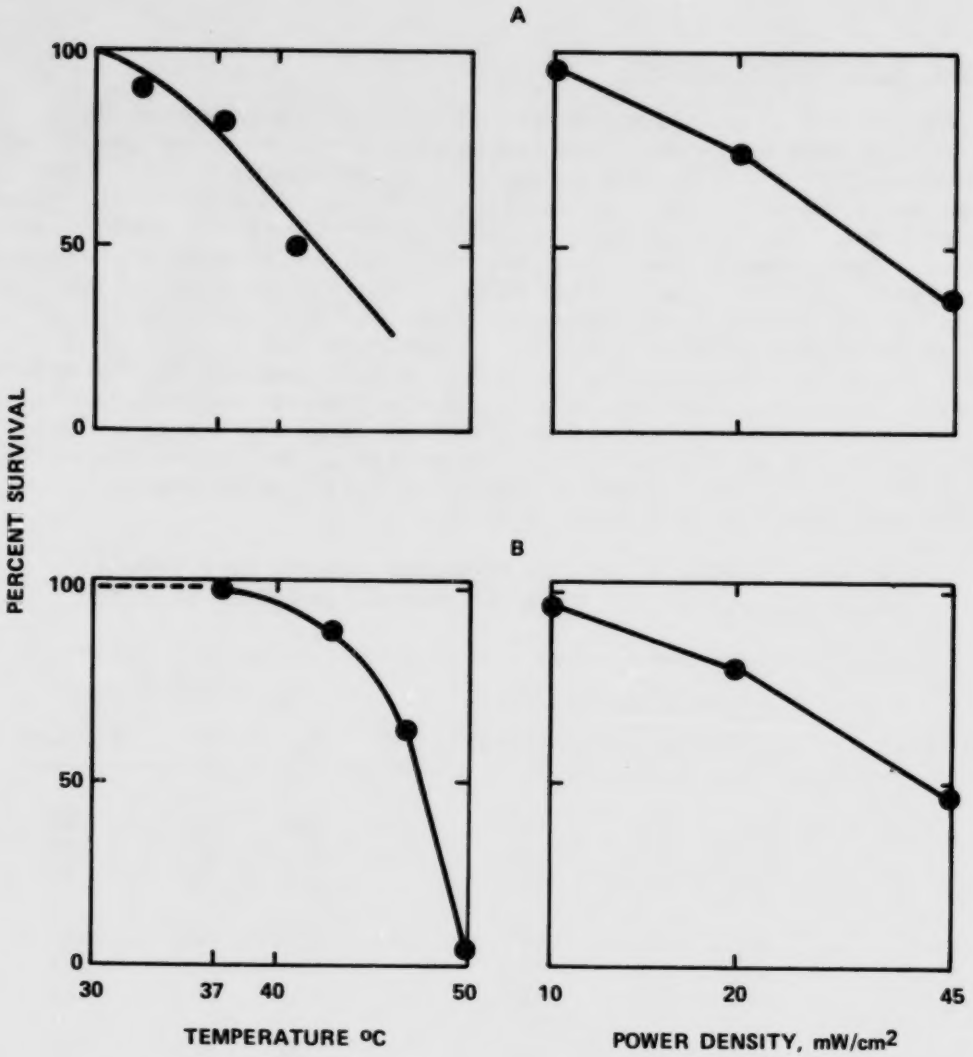


Figure 4. Survival curves of yeast and bacterial cultures when exposed for 1.5 to 2.0 hours to either elevated temperatures or microwave radiation. The percent cell survivals were calculated by comparing the respective colonies grown in the sham (control) conducted at 30°C (for yeast) and 37°C (for bacteria) in each test. Figure A represents cell survival when the yeast, Sacch. cerevisiae strain D_4, was exposed to elevated temperature as well as to different power densities of 8.8 GHz pulsed radiation. Similarly, Figure B shows such survival curves with TA-100 strain of the bacterium, S. typhimurium.

Effects in Waveguide Exposure

The effects of pulsed microwave radiation on the growth of the E. coli strains were explored. Experiments were performed under controlled temperature (37° ± 0.1°C) in a waveguide exposure system using 8.6 GHz pulsed radiation which had a pulse width of 1 μsec and a pulse repetition rate of 1 KHz. The average specific absorption rate of the sample was 12 W/kg. Two parameters were investigated: (1) the effects of microwave radiation on actively growing (log phase) E. coli strains pol A^+ and pol A^- and (2) the effect of microwave radiation on cells maintained in a non-nutritive salt solution (saline). Exposure of the E. coli pol A^+ strain for up to 7 hours did not stimulate growth compared to sham-exposed controls. Similarly, the data did not show microwave-induced growth of E. coli pol A^-, although a trend was observed ($0.05 < P < 0.1$). The E. coli strains that were irradiated in saline for up to 7 hours showed no change in the subsequent growth of normal or repair deficient bacteria. Results are summarized in Tables 10 to 12.

TABLE 10. COMPOSITE DATA OF MICROWAVE INDUCED CELL GROWTH OF E. COLI POL A^+ AND POL A^- IN VARIOUS INTERVALS OF TIME

	Pol A^+			Pol A^-		
Time (hrs)	N_o*	N_I†	N (sham)§	N_o	N_I	N (sham)
1	151	157	137	130	146	131
1	130	132	128	140	169	174
1	141	144	140	140	187	190
2	147	195	184	149	200	183
2	131	179	177	129	177	183
2	146	187	181	113	154	155
4	142	237	242	122	271	250
4	149	238	259	141	286	281
4	129	213	236	116	275	247
7	109	234	245	115	292	304
7	111	284	286	137	395	342
7	115	293	279	124	267	312

*Initial number of colonies in 0.5 ml of diluted culture.

†Number of colonies after exposure in 0.5 ml of diluted culture.

§Number of colonies after sham treatment in 0.5 ml of diluted culture. Sham experiments were conducted under identical conditions except without microwave exposure.

TABLE 11. REGRESSION ANALYSIS OF COMPOSITE DATA ON MICROWAVE RADIATION

Strain	Treatment	Regression coefficient (k)	R^2
A^+	control	0.126 ± 0.014	0.977
A^+	sham	0.124 ± 0.015	0.969
A^+	irradiated	0.124 ± 0.011	0.984
A^-	control	0.141 ± 0.012	0.986
A^-	sham	0.144 ± 0.017	0.973
A^-	irradiated	0.149 ± 0.022	0.947

Regression coefficients (k) are obtained by computer linear regression analysis of experimental data. The limits represent the 95 percent confidence interval. K value represents the relative growths of pol A^+ and pol A^- strains of E. coli. For best fitting of the curve, the square of the multiple correlation coefficients (R^2) should be close to 1.0.

TABLE 12. SUMMARY OF CALCULATED REPAIR INDICES AT DIFFERENT TIME INTERVALS

Time (hours	Repair index values treatment	
	Irradiated	Sham
1	1.010	1.006
2	1.020	1.011
4	1.042	1.023
7	1.075	1.041

Repair index values with respect to control were calculated using Eqs. (3) and (4) as described in the text. K values in Eqs. (3) and (4) are obtained by curve fitting of the experimental data using a SAS (Statistical Analysis System) computer program in order to generate the best possible values for the cell population.

Exposure of E. coli Cells to Extremely Low-level Microwave Intensities Using Various Exposure Systems

Table 13 summarizes results of exposure of E. coli cells to 8.6 GHz, 8.8 GHz, and 9 GHz for varying time of exposures at extremely low power using a far-field antenna exposure system. No microwave effect was apparent when compared to sham treatments.

Table 14 summarizes results obtained by exposure of E. coli cells to 10 GHz pulsed microwave radiation using a waveguide at an SAR of 0.1, 1, or 10 mW/g. No microwave effects were apparent when compared to sham treatments.

Tables 15 and 16 summarize data when E. coli pol A^+ and pol A^- strains were exposed to 0.1, 1.0, or 5.2 mW/g power levels using a Crawford cell at 915 MHz. Calculated repair-indices did not show any radiation effect at any of the time intervals (Tables 15, 16, and 17).

TABLE 13. SUMMARY OF CELL GROWTH RATIOS IN TERMS OF COLONY FORMING UNITS (CFU) OF E. COLI POL A^+ STRAIN AT 8.6, 8.8, AND 8.9 GHz USING A FAR-FIELD ANTENNA EXPOSURE SYSTEM AT 1 mW/cm²

Hours of exposure	8.6 GHz		8.8 GHz		9 GHz	
	N (irr)* / No§	N (sham)† / No	N (irr) / No	N (sham) / No	N (irr) / No	N (sham) / No
1	2.19	2.25	2.37	2.03	2.03	2.10
1	2.88	3.15	2.46	2.06	2.32	2.05
1	2.60	3.06	2.47	1.89	2.01	1.73
5	4.03	4.12	3.47	3.05	4.74	4.69
5	3.22	2.96	3.51	3.18	3.68	4.26
5	3.90	3.23	3.66	3.15	4.18	4.06
10	5.58	5.72	5.25	5.47	5.41	4.55
10	5.13	5.21	5.01	4.73	5.28	4.86
10	5.33	4.98	5.16	5.31	5.34	5.03
15	7.56	7.15	7.27	7.78	6.61	7.05
15	7.28	7.76	7.12	6.86	6.75	6.37
15	7.09	6.84	6.84	7.15	6.24	6.57

*No. of cells after irradiation.

†No. of cells in control tests.

§No. of cells at initial time (zero time exposure).

TABLE 14. SUMMARY OF CELL GROWTH RATIOS OF *E. COLI* POL A$^+$ AT 10 GHz PULSED RADIATION

Hours of exposure	0.1 mW/g		1 mW/g		10 mW/g	
	N (irr)* / No§	N (sham)† / No	N (irr) / No	N (sham) / No	N (irr) / No	N (sham) / No
7	4.91	4.74	2.66	2.86	5.01	5.17
7	5.23	5.13	3.23	3.48	4.69	4.78
7	4.78	4.49	3.35	3.23	4.88	4.67
15	8.00	7.81	6.33	6.03	7.37	7.56
15	8.01	7.14	10.89	10.03	6.82	7.48
15	7.03	7.13	8.97	9.45	7.76	7.43

*No. of cells after irradiation.

†No. of cells in control tests.

§No. of cells at initial time (zero time exposure).

TABLE 15. SUMMARY OF REPAIR INDICES AT DIFFERENT DOSE RATES

Dose rate (mW/g)	Repair Index			
	2 hours	4 hours	7 hours	15 hours
0.1	1.12	1.04	0.94	1.02
1.0	0.96	1.01	0.98	1.03
5.2	1.12	0.99	1.11	1.05

TABLE 16. SUMMARY OF CELL GROWTH OF E. COLI POL A^+ STRAIN AT VARYING DOSE RATES OF 915 MHz CW RADIATIONS USING A CRAWFORD CELL SYSTEM

Time of Exposure (Hours)	0.1 mW/g $\frac{N\ (pol\ A^+)}{No\ (pol\ A^+)}$	1 mW/g $\frac{N\ (pol\ A^+)}{No\ (pol\ A^+)}$	5.20 mW/g $\frac{N\ (pol\ A^+)}{No\ (pol\ A^+)}$	37°C $\frac{N\ (pol\ A^+)}{No\ (pol\ A^+)}$
2	2.66	3.09	2.18	2.43
2	2.42	2.18	3.03	3.30
2	2.55	2.59	2.58	2.65
4	4.09	3.94	3.60	4.42
4	3.54	5.41	4.25	4.04
4	4.29	4.00	4.45	4.24
7	4.67	4.66	4.09	4.61
7	4.93	4.93	3.57	4.16
7	4.75	4.73	4.86	4.82
15	7.92	7.86	8.76	9.10
15	7.45	7.07	7.01	7.02
15	7.60	7.59	7.64	7.10

No (pol A^+) = Number of cells of E. coli pol A^+ strain in 0.5 ml of culture with 10^6 times dilution using 0.9% saline at time zero.

N (pol A^+) = Number of cells of E. coli pol A^+ strain in 0.5 ml of culture with 10^6 times dilution using 0.9% saline following exposure for any specified time.

TABLE 17. SUMMARY OF CELL GROWTH OF E. COLI POL A⁻ STRAIN AT VARYING DOSE RATES OF 915 MHz CW RADIATIONS USING A CRAWFORD CELL SYSTEM

Time of Exposure (Hours)	0.1 mW/g N (pol A⁻) / No (pol A⁻)	1 mW/g N (pol A⁻) / No (pol A⁻)	5.20 mW/g N (pol A⁻) / No (pol A⁻)	37°C N (pol A⁻) / No (pol A⁻)
2	2.61	2.37	3.18	2.96
2	3.07	2.17	3.29	2.78
2	2.98	3.09	2.32	2.65
4	3.75	4.38	3.73	4.36
4	4.71	5.61	4.58	4.76
4	4.27	3.77	4.21	3.88
7	4.75	5.55	5.58	4.35
7	4.62	5.06	4.88	5.50
7	5.76	4.85	4.98	5.20
15	9.32	9.22	11.64	8.90
15	8.68	8.33	8.06	8.86
15	8.91	9.04	8.44	8.83

No (pol A⁻) = Number of cells of E. coli pol A⁻ strain in 0.5 ml of culture with 10^6 times dilution using 0.9% saline at time zero.

N (pol A⁻) = Number of cells of E. coli pol A⁻ strain in 0.5 ml of culture with 10^6 times dilution using 0.9% saline following exposure for any specified time.

ANCILLARY STUDIES

There is a fundamental problem in microwave research which must be addressed. That is, what is the confunding influence of microwave-induced temperature rise and how can the influence of this temperature rise be removed from the overall change in the biological endpoint. One approach is to observe the response to various temperatures and compare the predicted response at various temperatures with the response observed under microwave exposure. With this in view, we performed several extensive studies. Results of one of those studies are described below.

A Quantitative Comparison of Thermal Versus Microwave-induced Alterations in Bacterial Growth

An interpretation of microwave-induced biological effects is often confused due to the inability to discount the thermal response component from the total response of biological systems. A technique has been described to quantitatively analyze bacterial growth responses associated with thermal and microwave treatments using E. coli pol A^+ and pol A^-. Data for the thermal response models were obtained by exposing bacterial cultures in 35 mm diameter petri dishes to a series of temperatures (37°C to 49°C) for various times (1 hour to 15 hours). Data for the microwave response were obtained by exposing similarly-prepared cultures at one temperature (35°C) to 8.8 GHz radiation pulsed at 1000 MH with a duty cycle of 0.001 and a SAR of 40 W/kg. Knowledge of the temperature of the microwave-exposed samples allows a comparison of the microwave growth rate with the thermal growth rate at the same temperature. The difference between the observed and the thermal growth rates is then tested for significance by using the two-way analysis of variance. In this case, the bacterial growth response could be completely described by the thermal response. The basic approach described here should prove useful wherever a microwave-induced change is to be evaluated when endpoint is susceptible to temperature perturbation. Results are summarized in Tables 18 to 20 and Figure 5.

TABLE 18. SUMMARY OF CELL GROWTH OF E. COLI POL A⁻

Time of exposure (hours)	Exposed to 8.8 GHz N_o*	N†	N/N_o	Time (hours)	Exposed at 35.2°C N_o	N_c	N_c/N_o
2	120	167	1.392	1	168	185	1.074
2	122	177	1.451	1	168	179	1.068
2	157	221	1.408	1	168	185	1.098
5	141	204	1.447	5	168	302	1.795
5	140	194	1.386	5	168	309	1.942
5	143	207	1.448	5	168	312	1.857
10	162	187	1.154	10	168	593	3.529
10	110	128	1.164	10	168	551	3.280
10	114	141	1.237	10	168	562	3.348
15	142	147	1.035	15	92	628	6.126
15	146	202	1.062	15	122	701	5.746
15	126	130	1.032	15	122	751	6.205

*Number of cells at time zero in 0.5 ml of culture diluted 10^6 by 0.9% saline.

†Number of cells at any specified exposure time in 0.5 ml culture diluted 10^6 by 0.9% saline.

TABLE 19. SUMMARY OF CELL GROWTH OF *E. COLI POL* A^+

Time of exposure (hours)	Exposed to 8.8 GHz N_o*	N†	N/N_o	Time (hours)	Exposed at 35.2°C N_o	N_c	N_c/N_o
2	151	217	1.436	1	149	155	1.040
2	149	211	1.416	1	149	152	1.017
2	144	196	1.361	1	149	161	1.081
5	140	201	1.436	5	149	271	1.818
5	144	209	1.451	5	149	265	1.781
5	142	199	1.401	5	149	261	1.755
10	123	154	1.250	10	149	481	3.231
10	120	161	1.342	10	149	462	3.101
10	127	159	1.252	10	170	570	3.353
15	128	134	1.047	15	110	530	4.840
15	127	141	1.110	15	112	576	5.129
15	140	171	1.221	15	112	566	5.054

*Number of cells at time zero in 0.5 ml of culture diluted 10^6 by 0.9% saline.

†Number of cells at any specified exposure time in 0.5 ml culture diluted 10^6 by 0.9% saline.

TABLE 20. SUMMARY OF THE CELL GROWTH AT 42.2°C OF *E. COLI POL* A^+ AND *POL* A^- STRAINS

Time (hours)	N_o (*pol* A^+)	N (*pol* A^+)	$\frac{N \text{ (pol } A^+)}{N_o \text{ (pol } A^+)}$	N_o (*pol* A^-)	N (*pol* A^-)	$\frac{N \text{ (pol } A^-)}{N_o \text{ (pol } A^-)}$
2	232	279	2.356	123	177	1.439
2	115	154	1.339	123	173	1.407
2	120	167	1.392	109	159	1.459
5	128	183	1.426	121	171	1.412
5	132	186	1.409	115	164	1.422
5	132	197	1.492	115	166	1.443
10	115	151	1.313	123	149	1.211
10	156	173	1.109	123	146	1.187
10	120	139	1.158	160	201	1.256
15	156	166	1.064	121	135	1.116
15	120	123	1.025	121	132	1.092
15	156	162	1.038	160	175	1.074

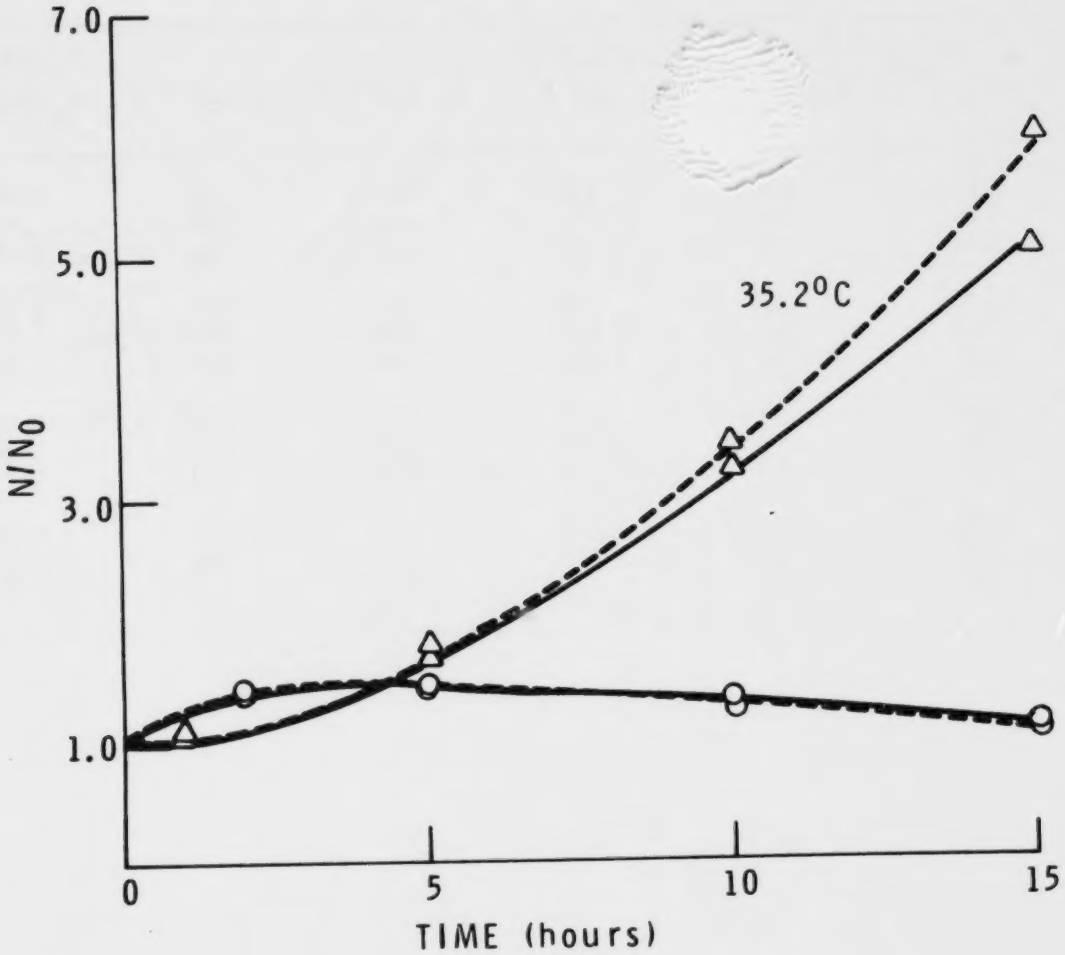


Figure 5. Composite data showing plot of average N/N_o against time of exposure.

N = Number of cells at specified time
N_o = Number of cells at time zero

Growth of cells N/N_o treated at 35.2°C in air incubator is shown by Δ--Δ and O--O represents survival of cells treated with 8.8 GHz pulsed microwave radiations in a anechoic exposure chamber at 35.2° ± 0.2°C. The specific absorption rate (SAR) is 40 W/kg.

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